Application of Quantitative Microbial Risk Assessments for Estimation of Risk Management Metrics: *Clostridium perfringens* in Ready-to-Eat and Partially Cooked Meat and Poultry Products as an Example[†]

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ABSTRACT

The U.S. Department of Agriculture, Food Safety and Inspection Service is exploring quantitative risk assessment methodologies to incorporate the use of the Codex Alimentarius' newly adopted risk management metrics (e.g., food safety objectives and performance objectives). It is suggested that use of these metrics would more closely tie the results of quantitative microbial risk assessments (QMRAs) to public health outcomes. By estimating the food safety objective (the maximum frequency and/or concentration of a hazard in a food at the time of consumption) and the performance objective (the maximum frequency and/or concentration of a hazard in a food at a specified step in the food chain before the time of consumption), risk managers will have a better understanding of the appropriate level of protection (ALOP) from microbial hazards for public health protection. We here demonstrate a general methodology that allows identification of an ALOP and evaluation of corresponding metrics at appropriate points in the food chain. It requires a two-dimensional probabilistic risk assessment, the example used being the Monte Carlo QMRA for *Clostridium perfringens* in ready-to eat and partially cooked meat and poultry products, with minor modifications to evaluate and abstract required measures. For demonstration purposes, the QMRA model was applied specifically to hot dogs produced and consumed in the United States. Evaluation of the cumulative uncertainty distribution for illness rate allows a specification of an ALOP that, with defined confidence, corresponds to current industry practices.

Traditionally, U.S. Department of Agriculture, Food Safety and Inspection Service (FSIS)–regulated food processing systems relied on process control and meeting performance standards to achieve safe and wholesome products. Until the recent utilization of quantitative microbial risk assessments (QMRAs), such measures could not be directly linked to public health outcomes, so the impact of such efforts was not easily estimated. However, the use of QMRAs allows linking of public health outcomes due to consumption of contaminated products with the microbiological status of the products within the food chain *(6)*.

In its desire to improve human health and the sanitary situation in member countries, the World Trade Organization agreed to sanitary and phytosanitary measures (the SPS Agreement) including the introduction of the appropriate level of protection (ALOP; the level of protection deemed appropriate by the member establishing a sanitary or phytosanitary measure to protect human, animal, or plant life or health within its territory), which is based on an assessment appropriate to the member's circumstances *(4)*. The Codex Alimentarius recently adopted guidelines for risk management *(2),* which attempt to link the ALOP to microbiological metrics in the food chain up to the point of consumer consumption. These metrics include traditional metrics such as the microbiological criterion (MC) with its associated sampling plans (an MC for food defines the acceptability of a product or a food lot, based on the absence or presence, or number of microorganisms including parasites, and/or quantity of their toxins and metabolites, per unit[s] of mass, volume, area, or lot *(1)*) and newer metrics such as the food safety objective (FSO) and the performance objective (PO) *(2).*

While the United States is currently not using the newer metrics, there have been a few demonstrations of methodologies examining possible use of risk management metrics, including consideration of quantified uncertainties (e.g., *(7, 12–15)*). For the most part though, those attempts to apply the methodology appear to lack sufficient characterization of uncertainty for practical application. We here demonstrate that a more detailed characterization of uncertainty is sufficient for the practical application of

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quantitative microbial risk assessment as an approach to implement the newer risk management metrics adopted by the Codex Alimentarius.

To demonstrate an approach to estimate risk management metrics that may be linked to public health outcomes, we chose to use a recently completed quantitative microbial risk assessment involving *Clostridium perfringens (3, 5). C. perfringens* is an anaerobic bacterium widely distributed in the environment, which forms hardy spores in adverse conditions. The hazard to humans is from high concentrations of vegetative cells in food—when ingested, the vegetative cells sporulate, and some types of *C. perfringens* (type A CPE$^+$; CPE stands for ''*C. perfringens* enterotoxin'') produce a toxin during sporulation, which induces diarrheal illness. More severe illness can result in rare cases, but such cases are not examined here. The spores are resistant to cooking temperatures (greater than 55°C) that kill vegetative cells, but are stimulated to germinate into vegetative cells by such temperatures. Vegetative cells grow particularly rapidly at temperatures around 45°C, but may grow at all temperatures down to 12°C.

The *C. perfringens* risk assessment evaluates the effect on human illnesses of allowing different amounts of growth of *C. perfringens* during the critical ''stabilization'' (cool down) preparation step for ready-to-eat (RTE) and partially cooked foods containing meat or poultry after they are cooked (which cooking kills the vegetative cells, but stimulates the spores present in the meat to germinate). It is a two-dimensional (2-D) Monte Carlo QMRA that tracks *C. perfringens* vegetative cells and spores in individual servings of RTE and partially cooked foods from their initial production until final consumption. The primary variable contributing to human illnesses was storage of RTE or partially cooked foods at elevated temperatures (greater than 12°C) during commercial or home storage. For the small fraction of servings that were contaminated with *C. perfringens* vegetative cells and that were also stored at relatively high temperatures, *C. perfringens* vegetative cell counts highly likely to cause human illness could easily be achieved.

The feature of the *C. perfringens* risk assessment that makes it suitable as a demonstration for the methodology examined here is its incorporation of a 2-D (variability and uncertainty) probabilistic approach. To simplify this demonstration, a single RTE or partially cooked food type, the hot dog, was chosen because of its high consumption rate and its ease of use within the *C. perfringens* risk assessment model. Hot dogs contributed between 15 and 19% of the estimated total illnesses caused by *C. perfringens* from all RTE and partially cooked foods (at the maximum likelihood estimate for uncertainty). However, various simplifying assumptions made in the risk assessment mean that the estimates obtained in this demonstration may not be representative of the U.S. hot dog industry—the values obtained here should not be considered authoritative, but merely demonstrative of the approach that may be used with fully representative data. This particular QMRA is used here solely as a vehicle to demonstrate the practicality of the methodology.

For the demonstration, the FSO is defined as a more general specification, using arbitrary surrogate measures, and we demonstrate how a 2-D QMRA allows evaluation of an FSO that provides the ALOP with required confidence. A PO may be defined at any point in the food chain, and the same approach to evaluation as for the FSO may be followed, provided the QMRA is suitably specified. The QMRA allows exploration of various specifications for a PO, selection of the most practical or efficient, and an evaluation that provides the ALOP with required confidence. Finally, given a PO, an MC may be defined by using standard approaches to sampling and experimental design.

MATERIALS AND METHODS

Selection of an ALOP. In principle, establishment of an ALOP could depend on the appropriate level of protection that is considered desirable by a country within its territory; however, any such ALOP would have to be binding both internally and externally, and countries would have to enforce it within their territory for it to be applicable in international trade. In practice therefore, current conditions within a country are considered acceptable, and it would usually be desirable to establish an ALOP at as low a level as possible while allowing current practices. We reiterate that no ALOP (for *C. perfringens* in RTE foods) has been currently established by the United States.

This demonstration proceeds by considering how an ALOP might be established as low as possible while corresponding to current conditions in the United States, assuming that the *C. perfringens* QMRA accurately reflects those current conditions and describes the uncertainty about current conditions (with all the approximations that entails).

We apply the *C. perfringens* risk assessment model *(5)* to hot dogs alone. Figure 1a and 1b shows (uncertainty) maximum likelihood estimates from the QMRA over a range of allowable log *C. perfringens* growth during the critical stabilization step of manufacture. For conditions currently considered representative (a growth of 1 log during stabilization), the QMRA estimates 3.6 illnesses per million hot dog servings, and a prevalence of 5.0 per 1,000 servings having a non-zero number of CPE$^+$, type A *C. perfringens* spores or vegetative cells at the time of consumption (Fig. 1a and 1b). Assuming no variance from the critical stabilization step, the 1-log growth is considered acceptable for the purposes of this analysis. Here and throughout the entire analysis, *prevalence* implies detection using standard methods, with the implicit assumption that the probability for detection is sufficiently constant that variations in that probability are inconsequential.

At 1-log growth during stabilization, running the *C. perfringens* risk assessment model allows evaluation of the uncertainty distribution of the illness rate (Fig. 2). From this distribution, one can read off the lowest illness rate that with any given confidence is currently achieved (insofar as the risk assessment model correctly codifies the uncertainties; the risk assessment should be consulted for further details about unknown uncertainties *(3)*). From the results plotted in Figure 2, the 95th percentile point of the uncertainty distribution (normal ordinate, the point on the cumulative standard normal distribution corresponding to this percentile, of 1.645) is at 13 illnesses per million servings, and the 99th percentile point (normal ordinate of 2.326) is at 21 per million. Thus, insofar as the risk assessment model reflects reality, establishing an ALOP of 21 illnesses per million servings would result in a 1% chance (or less) that current
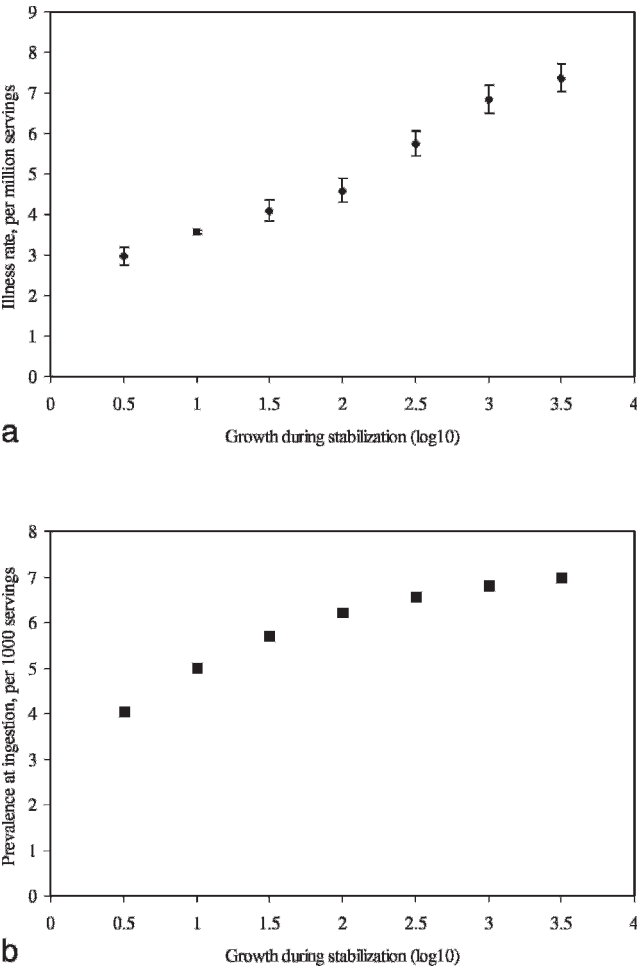
FIGURE 2. *Cumulative uncertainty distribution for the illness rate at 1-log growth.*

FIGURE 1. *(a) Illness rate and (b) prevalence of live CPE$^+$, type A* Clostridium perfringens *vegetative cells versus growth during stabilization. Error bars correspond to an 80% binomial confidence range. At 1-log growth, 30 times as many servings were simulated as at other growths.*

conditions fail the ALOP, and establishing an ALOP of 13 illnesses per million servings would result in a 5% chance (or less) that current conditions fail the ALOP.

Estimating an FSO: general considerations. The Codex Alimentarius' definition of an FSO is ''the maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the appropriate level of protection (ALOP).'' *Concentration* strictly is a mass/unit volume, whereas the most relevant hazard metric may well be different—for example, for *C. perfringens* in hot dogs, the natural metric to use might be number per hot dog or per food serving. *Frequency* is strictly defined to be per unit time, but clearly, what is meant is per unit of food (prevalence). Here, the natural unit is prevalence in servings (fraction of servings that are contaminated), or prevalence in hot dogs (fraction of hot dogs that are contaminated); other definitions (e.g., the prevalence in 10-g samples) might be more useful in other circumstances (e.g., for an MC). Others also suggest that other considerations be taken into account to derive an FSO (e.g., frequency and amount of food consumption, variability of pathogen concentration, prevalence of contaminated food) *(7, 9)*.

Suppose the ALOP is specified in terms of some (adverse) response metric *R,* which is derived from the frequency distribution
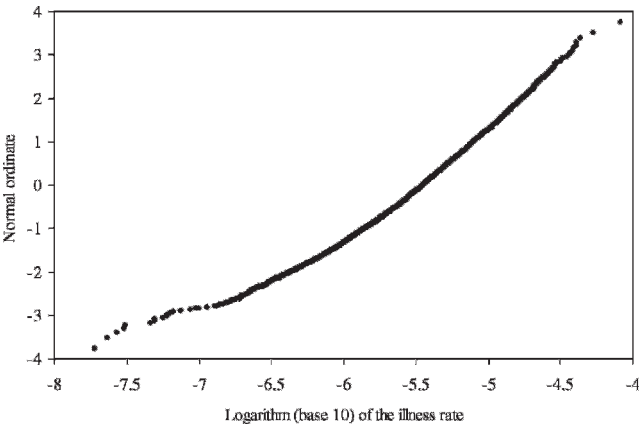
$q(r)$ of some response r for all food servings (a variability distribution; it is straightforward to examine unit masses of food rather than food servings). It is assumed here that the probability for the adverse event(s) defining the ALOP is a monotonic function of the evaluated response r. Then, without loss of generality, the probability for the adverse event(s) for which the ALOP is defined may be evaluated and used as a response; this restriction is used in what follows, so the evaluated response r is subsequently the probability for the adverse event used to define the ALOP. The variability distribution $q(r)$ for this response may contain discrete parts (e.g., it may contain finite probability mass at zero and other probabilities) so all integrals shown below should be interpreted in the Lebesgue or more general sense, or equivalently the discrete parts of the distributions should be handled separately.

The most common examples for R are likely to be

$$R = \max r$$
(maximum response)   (1a)

$$R = \int rq(r)\,dr$$
(average response)   (1b)

$$R = \int_{r>0} q(r)dr$$
(prevalence of any response)   (1c)

$$R = \int_{r>r_1} q(r)dr$$
(probability of a response larger than $r_1 \geq 0$)   (1d)

For a common hazard, or for an acute toxic agent with a well-defined threshold for all members of the population, one may wish to specify an ALOP so that the probability for an adverse event is always sufficiently small for every individual food serving—so the maximum probability (equation 1a) for the adverse event arising from the hazard is limited for every serving. Even for an acute toxic agent with a well-defined threshold, it is unlikely that an ALOP could be set that is guaranteed safe for every member of the population, because of the possibility for allergy or other abnormal sensitivity. The same is true even for food components generally considered nontoxic and nutritious, e.g., peanuts.

For an organism causing a non–life-threatening illness, the average response (equation 1b) may be relevant—the ALOP may be specified by the illness rate, expressed for example as the

expected number of illnesses per million servings. If the adverse outcome is always life threatening, it may be appropriate to specify the ALOP purely in terms of the prevalence (equation 1c) of the response. A more general approach is to specify the ALOP in terms of the prevalence of servings with probability of response exceeding some threshold (equation 1d).

Now suppose the dose-response relation between the response r and a hazard metric h applicable to the food of interest is

$$r = f(h) \tag{2}$$

so that, at hazard metric h there is a probability $f(h)$ for the adverse event to occur (as stated above, the response measure is selected as the probability for the adverse event defining the ALOP). Suppose also that the frequency of occurrence of hazard metric h among food servings is specified by a probability distribution g (the variability distribution for h). Then the four examples above give, respectively,

ALOP   FSO

$R = \max r =$ $\max f(h)$

                        (maximum h if f is monotonic)   (3a)

$R = \int rq(r)dr =$ $\int f(h)g(h)dh$

                        (an average)   (3b)

$R = \int_{r>0} q(r)dr =$ $\int_{f(h)>0} g(h)dh$

                        (a prevalence)   (3c)

$R = \int_{r>r_1} q(r)dr =$ $\int_{f(h)>r_1} g(h)dh$

                        (a generalized prevalence)   (3d)

The right-hand side of the above equations demonstrate how these ALOP specifications may be translated to an FSO, based on the hazard metric h. The first case (equation 3a) corresponds to using the maximum hazard metric (assuming the dose-response relationship is monotonic) in the FSO. The second case (equation 3b) requires an average that cannot be expressed in terms of prevalence and/or maximum hazard metric; the entire distribution for the hazard metric is required, together with the dose-response relationship. The third case (equation 3c) corresponds to using the prevalence of any non-zero response (for a non-threshold dose-response, this is the prevalence for any non-zero hazard metric). The fourth case (equation 3d) requires a generalized prevalence measure for the hazard metric. In the third and fourth cases, one could redefine the hazard metric to account for any threshold (e.g., concentration above a threshold concentration, or more likely, quantity per serving above a threshold quantity), in which case the FSO could be defined purely in terms of the prevalence of the redefined hazard metric. However, such an approach would likely become complicated when attempting to account for variability and uncertainty in any such threshold.

The specification of the ALOP therefore induces a requirement on the functional of the distribution g that must be controlled to achieve the ALOP. However, the particular functional R required by the ALOP specification may not be readily measurable, so that it may be desired to use a surrogate. For example, it may be strictly required to use the integral from equation 3b to specify an FSO when the ALOP is specified in terms of the expected number of illnesses per serving, but this integral may be difficult to measure. Instead, the FSO may be defined in terms of some

surrogate S that is easier to measure (e.g., prevalence, mean contamination level, maximum contamination level).

The problem with the use of a surrogate S is that knowledge of S does not imply complete knowledge of R, so that introducing a surrogate introduces extra uncertainty. In such a case, specification of the FSO changes from:

$$\text{select } R \text{ such that } R < \text{ALOP} \tag{4}$$

to:

$$\text{select the boundaries of } S \text{ such that } \Pr(R > \text{ALOP}|S) \le \alpha \tag{5}$$

where α is an acceptable level of risk to exceed the ALOP. That is, the surrogate S has to be chosen so that the conditional probability for R to exceed the ALOP given S is less than the acceptable level of risk to exceed the ALOP. The range of values of S obtained from equation 5 is that which is compatible with the ALOP and the acceptable level of risk to exceed the ALOP.

Equation 5, rather than equation 4 is that most likely to be used, in that usually R is not known exactly, so that even in the best case one really obtains a surrogate S for R, although S may just be R with uncertainty added. However, equation 5 covers the case for any surrogate, not just the case where the only degree of surrogacy occurs because of uncertainty.

The selection of a level of risk (the probability α in equation 5) has to be made with some care, since it may interact with the establishment of an ALOP based on current conditions in a way that is not immediately obvious. The method of establishing an ALOP given above results in some small chance (β) that current conditions fail to meet that ALOP. If an FSO is now selected in such a way that there is only some small probability α for conditions corresponding to that FSO to fail the ALOP, then if $\alpha = \beta$, the FSO will essentially conform to current conditions. However, if $\alpha < \beta$, then the FSO may be more stringent than current conditions, whereas if $\alpha > \beta$, the FSO is likely to be less stringent than current conditions.

Using a risk assessment model. Evaluation of an FSO for some surrogate S as in equation 5 may be accomplished by using a 2-D probabilistic risk assessment model that accounts for both variability (e.g., between food servings, leading to the variability distribution for the hazard metric h and response r) and uncertainty. Such assessments typically operate by computing the variability distribution for h and r in an inner loop (with fixed selections from the uncertainty distributions), and the uncertainty distribution in an outer loop (in which different selections are made from the uncertainty distributions), and this approach will be assumed here. The nomenclature used here will correspond to Monte Carlo analyses, but the same ideas may be used in the less common instances where analytic methods are used for the variability distribution, the uncertainty distribution, or both.

After constructing the variability distribution for each selection from the uncertainty distributions, the values of R and S can be computed (since within the risk assessment model all quantities are known). For example, in the risk assessment model, the dose-response function f will have uncertain parameters θ, with best estimates Θ, and the frequency distribution g will have uncertain parameters ϕ (there will usually be little or no overlap between θ and ϕ). Then, for example, if R is given by an average (equation 3b, above), in the risk assessment it may be evaluated for each selection from the uncertainty distributions as

$$R = \int f(h|\theta)g(h|\phi)dh \tag{6}$$

The surrogate chosen might be to use a best estimate of the dose-response function (using the best estimates of the parameters),
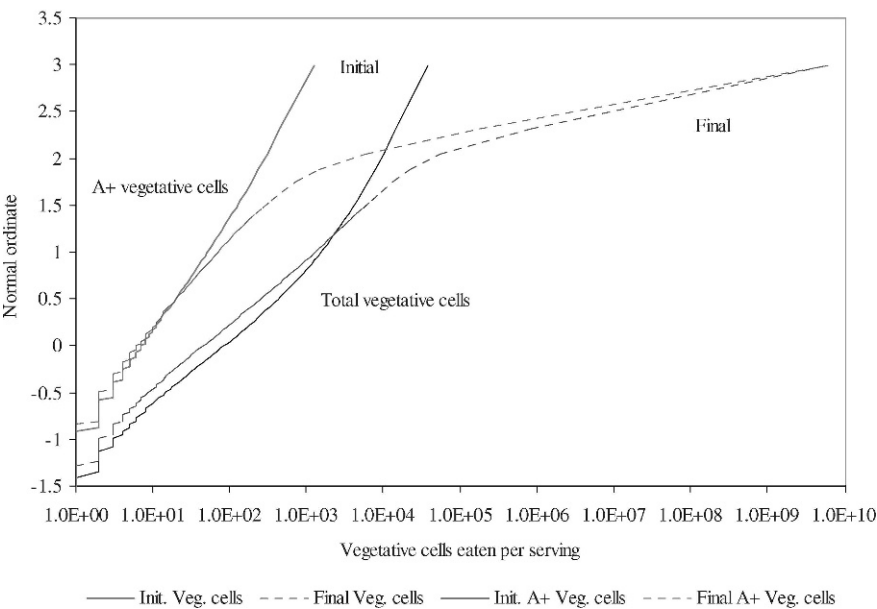
FIGURE 3. *Cumulative distributions for vegetative (Veg.) cell numbers (separately for total* Clostridium perfringens *and type A CPE* [+]; *Monte Carlo run with* 10^8 *servings) initially in the food serving (Init.) and at consumption (Final).*

which may be simultaneously evaluated as:

$$S = \int f(h|\Theta)g(h|\phi)dh \qquad (7)$$

Thus, both S and R may be computed from each variability distribution, and the resulting set of S and R values obtained from making different uncertainty distribution selections (the uncertainty loop) may be used to construct the confidence region specified by equation 5.

In practice, it will often be assumed that there is a common uncertainty distribution for the ratio of R to S (in principle, the uncertainty distribution for R could depend on the value of S in more complex ways), so this ratio may be computed for each uncertainty iteration, allowing construction of the uncertainty distribution for this ratio. Confidence limits on the ratio then allow construction of the region of S specified by equation 5. That is the approach taken here. As usual in Monte Carlo assessments, some care has to be taken in choosing the numbers of samples in order to achieve numerical stability.

The ALOP for *C. perfringens* evaluated above corresponds to equation 1b. Strictly, evaluation of an FSO corresponding to that ALOP must take account of the full distribution of hazard metric (in this case, the number of *C. perfringens* type A CPE^{+} vegetative cells in a serving) and the dose-response curve. In this case, to take account of the uncertainty in the dose-response curve, the specification of the FSO should be in the form:

$$\Pr\left(\int f(h)g(h)dh > \text{ALOP}\right) \le \alpha \qquad (8)$$

where α is the acceptable level of risk to exceed the ALOP (e.g., 1 to 5%). Since f is supposed not known exactly, equation 8 does not give an explicit practical method of evaluation; but one approach would be to define

$$S = \int f(h|\Theta)g_{obs}(h)dh \qquad (9)$$

where g_{obs} is the observed distribution of hazard index, so that S is computable from observations. Then knowledge of the uncertainty distribution of the ratio of R to S allows computation of the set $\{S\}$ specified by equation 5, that is

$$\{S\} \text{ such that } \Pr(R > \text{ALOP}|S) \le \alpha \qquad (10)$$

the boundaries of which set would be the FSO specification. In a Monte Carlo, the method of doing this would be to compute each

expression given in equations 6 and 7 in each uncertainty iteration, calculate the ratio R/S (using the assumption that the distribution of this ratio is independent of the value of S), and save those values. The resultant distribution of the ratio R/S may then be used to calculate the boundary of $\{S\}$ such that equation 10 is satisfied.

This discussion really presumes that g_{obs} can be specified in some useful parametric form, so that the set $\{S\}$ of equation 10 can be expressed in terms of those parameters. For example, if the shape of g_{obs} were known to be log normal, then it would be possible to evaluate the integral in equation 9 in terms of the median and geometric standard deviation, since those two parameters would then completely define g_{obs}, and then the boundary of the set $\{S\}$ in equation 10 would be defined by some inequality or inequalities relating those parameters to α, the acceptable level of risk to exceed the ALOP.

It turns out that no simple statistics (such as maximum concentration or prevalence) of the distribution $g(h)$ are strictly sufficient in the case of *C. perfringens* to adequately define an FSO, because specification of any such metric does not necessarily correlate (under all circumstances) with the frequency of illnesses (which is the desired metric for the ALOP). To illustrate, Figure 3 shows the (uncertainty) maximum likelihood estimate for cumulative distributions for numbers of vegetative cells initially present in the food servings (just prior to 10-fold growth during stabilization), and at the time of consumption. The initial numbers of cells are approximately log normal (with a slightly shorter tail, indicated by the upward curvature), whereas the final number of cells have a distribution that is difficult to describe compactly, but clearly has a very long right tail (at high vegetative cell numbers).

For *C. perfringens,* the predicted maximum number of cells in a serving is governed by the maximum vegetative cell concentration that *C. perfringens* can grow to in the food examined (in reality, it would be the maximum that would not be thrown out as evidently contaminated, a point evaluated in the risk assessment only in a sensitivity analysis). The risk assessment demonstrates that growth to such high concentrations is quite likely for a small fraction of food servings, because of the failed status of a small fraction of consumer refrigerators.

The small fraction of food servings so affected provides the long tail of the distributions seen in Figure 3. The importance of this long tail may be seen in Figure 4, which shows the servings

FIGURE 4. *Scatterplot of the ingested vegetative cell count versus the initial (vegetative plus spore) cell count for type A CPE$^+$ cells; all 1,878 cases with $>10^8$ cells per serving in 10^8 servings. The sharp left boundary is an artifact of approximations used in the model, but does not affect the discussions.*
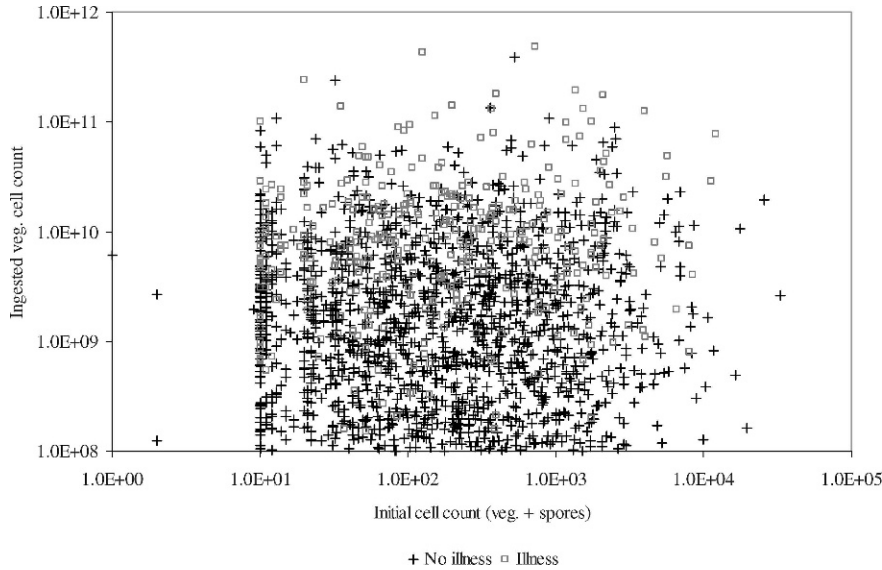
with large consumed cell counts ($>10^8$ cells per serving, at and to the right of the extreme right-hand end of Fig. 3) in this model run of 100,000,000 servings; servings causing illnesses are plotted as open squares. The selection of the cutoff cell count is such that Figure 4 contains approximately 97.4% (340 of 349) of the predicted illnesses, but only 0.0019% (1 in 53,000) of the total servings.

Thus, almost all the illnesses are due to the extreme right-hand end of the distribution in Figure 3, with the great majority of that distribution being irrelevant; only the top 0.0019% of the distribution contributes significantly.

Evaluation of the distribution $g(h)$ shown in Figure 3, and particularly the important part of that distribution with $>10^8$ cells per serving in this case requires the numerical approach of the risk assessment. Attempting to approximate the risk assessment model steps by using simple analytic distribution shapes for variability distributions (e.g., by assuming log normality of the cell number distribution), or using simple parameters (like geometric mean and standard deviation) for the whole distribution, would fail to correctly capture the critical part, the extreme upper tail of the final distribution. Although the particular details of this problem may be specific to *C. perfringens,* such details require separate evaluation in every case.

RESULTS

Evaluation of FSO for *C. perfringens*: using prevalence as a surrogate. The number of illnesses produced by *C. perfringens* in the food servings examined is evidently directly proportional to the prevalence of *C. perfringens* in those food servings at the consumer, *all other things being equal*. Indeed, this should always be true for any food-related risk.

However, specifying an FSO in terms of prevalence requires the *assumption* (or a proof) that all other things would remain equal, in this case that changes in prevalence could be achieved without changing the distribution of cell counts in the servings still containing *C. perfringens*. It is not clear that this assumption holds. The risk assessment model demonstrates that only small changes in the number of illnesses can be achieved by modifying the allowed growth of germinated spores during stabilization after the lethality step of production. Figure 1 shows the changes in illness rate and prevalence, and they are clearly not proportional over the full range shown, so that changes in the distribution of cell counts must occur. However, directly modifying the prevalence would require different strategies, presumably designed to change the prevalence in the incoming raw material or elsewhere in the food supply chain. The effect of such strategies (with a fixed growth during stabilization) on the distribution of *C. perfringens* at the consumer is not known, and the risk assessment is not designed to evaluate such questions.

However, if one *assumes* that the distribution would not change, an FSO may be evaluated in terms of prevalence of type A CPE$^+$ *C. perfringens* in the final food servings.

The evaluation of an FSO under such conditions by using the risk assessment model is relatively straightforward. It is necessary to account for both the uncertainty in the dose-response assessment and, at fixed prevalence, the uncertainty in the distribution of numbers of vegetative cells in servings at the time they are eaten. In the risk assessment model, this simply requires evaluating the uncertainty distribution for the ratio of the illness rate to the prevalence. The ratio can be obtained for both type A CPE$^+$ and total *C. perfringens*. From this uncertainty distribution, and using the *assumption* of proportionality between illness rate and prevalence, the value of the FSO can be obtained at any particular uncertainty percentile by reading off the ratio of illness rate to prevalence, and then computing from this ratio the prevalence that corresponds to the chosen ALOP (which is specified as an illness rate). This sequence of operations corresponds to determination of the set $\{S\}$ in equation 5: here, the surrogate S is the prevalence of *C. perfringens* (either type A CPE$^+$ or total). The assumption of proportionality between R and S ensures that all uncertainties are correctly taken into account by evaluation of the distribution of the ratio of expected illness rate to prevalence, and provides a one-to-one correspondence between percentiles on this distribution and the corresponding boundary of $\{S\}$ (prevalence)—indeed, $\{S\}$ is a line segment, the upper boundary of which corresponds to risk level α to exceed the ALOP.
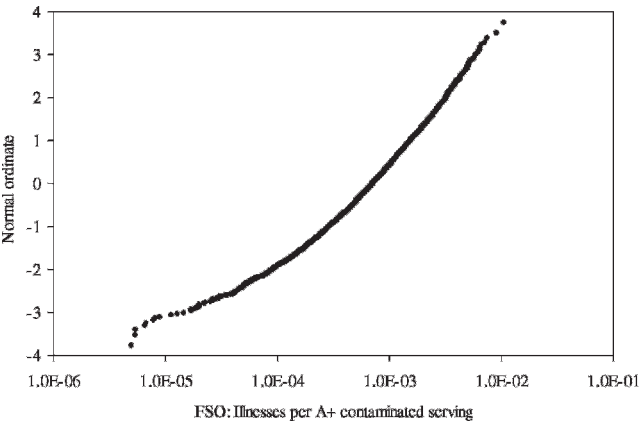
FIGURE 5. *Uncertainty distribution for the ratio of illness rate to vegetative* Clostridium perfringens *type A CPE$^+$ contamination rate at consumption.*

FIGURE 6. *Uncertainty distribution for the ratio of illness rate to total vegetative* Clostridium perfringens *(CP) contamination rate at consumption.*

Figure 5 shows the uncertainty distribution for the ratio of illness rate to *C. perfringens* type A CPE$^+$ vegetative cell contamination rate at consumption (this includes the effect of any cooking). The 95th percentile of this is at approximately 2.5×10^{-3}, and the 99th percentile is at approximately 3.8×10^{-3} illnesses per contaminated serving. A similar curve, Figure 6, may be constructed for the ratio of illness rate to *C. perfringens* total vegetative cell contamination rate at consumption. The 95th and 99th percentiles on these distributions are 1.2×10^{-3} and 1.8×10^{-3} illnesses per contaminated serving, respectively. Taking these two percentiles, and the two options for ALOP of 13 per million and 21 per million servings gives FSOs as shown in Table 1 (e.g., for total vegetative cell contamination at the 99th percentile and an ALOP of 13 illnesses per million servings, the FSO prevalence of contaminated servings is $13 \times 10^{-6}/1.8 \times 10^{-3} = 0.72\%$).

The general considerations discussed above should be borne in mind. The ALOP of 21 illnesses per million servings was designed so that there was 1% chance or less that current conditions exceeded that ALOP. Selecting an FSO so that there is only 5% chance the ALOP is exceeded (the 95th level of certainty) probably corresponds to an FSO that is less stringent than current conditions. Conversely, for an ALOP of 13 illnesses per million servings (5% chance or less that current conditions exceed that ALOP) an FSO selected at the 99th level of certainty will probably be more stringent than current conditions. The two choices with $\alpha = \beta$ result in nearly identical FSOs, and should correspond approximately to current conditions.
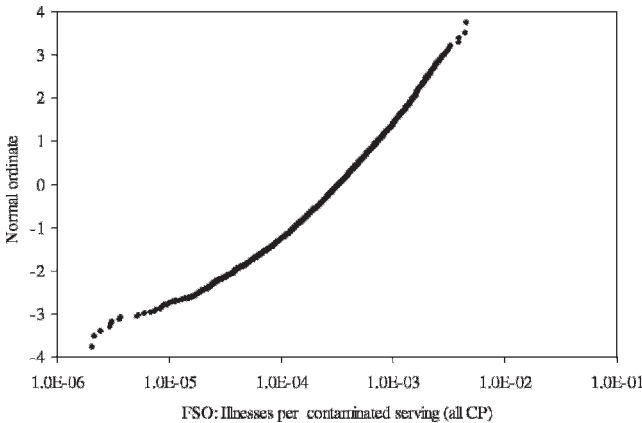
Evaluation of a PO for *C. perfringens* poststabilization. (i) A PO based on servings. The general considerations discussed above can be extended from evaluation of an FSO to the evaluation of a PO at an earlier point in the production of hot dogs. The selected point for this demonstration is immediately after the stabilization step, prior to any storage at (or transport from) the production location. For evaluation of a PO, some surrogate for the required functional R must necessarily be used, since prior to the point of consumption, the distribution $g(h)$ does not exist (except in the trivial case that there is no change in the distribution of hazard metric between the point of application of a PO and the point of consumption).

For *C. perfringens,* the risk assessment model allows evaluation of the relation between the distribution of numbers of cells per serving just after stabilization and just before consumption. Figure 7 shows this relationship for total *C. perfringens* for the 11,242 servings with non-zero vegetative cell number at consumption in a sample of 1,000,000 servings. A similar picture is obtained for type A CPE$^+$ *C. perfringens*. For relatively low final vegetative cell counts (less than about 10^6 cells per serving), there is a reasonable correlation between initial and final cell count. However, such low cell counts are very unlikely to produce illness. Most illnesses are caused by cell counts higher than 10^8 cells per serving (Fig. 4). Both Figures 4 and 7 (and other diagnostics) demonstrate that there is practically no correlation between initial and final numbers of cells for this extreme range—the distribution of cell counts $>10^8$ cells per serving is independent of the initial cell counts.

TABLE 1. *FSOs for the two ALOP selections and two levels of certainty*

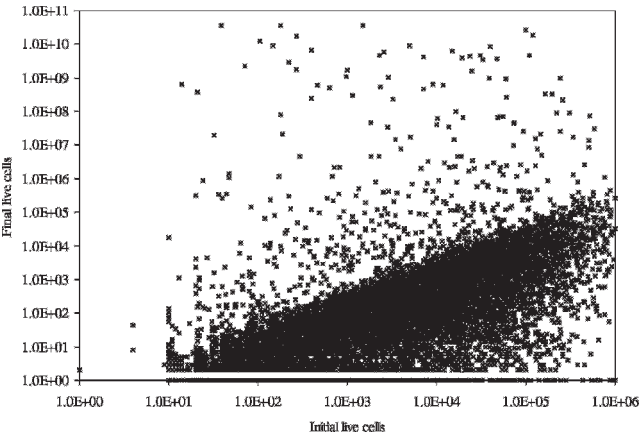| ALOP illnesses/ million servings	Level of certainty (percentile)	Prevalence in hot dog servings of:	
		Type A CPE$^+$ *C. perfringens* (%)	Total *C. perfringens* (%)
21	95th	0.85	1.80
	99th	0.55	1.16
13	95th	0.53	1.12
	99th	0.34	0.72

FIGURE 7. *Relationship between initial live cells (spores plus vegetative cells) and final vegetative cells, for non-zero final vegetative cells.*

FIGURE 9. *Uncertainty distribution for the ratio of illness rate to* Clostridium perfringens *contamination rate by total vegetative cells plus spores just after stabilization.*

Thus for the evaluation of a PO, using prevalence as a surrogate is probably about the best that can be done, since almost any other characteristic of the distribution of cells per serving just after stabilization appears to be irrelevant to the number of illnesses caused.

The approach is the same as for the FSO (the same Monte Carlo iterations can be used; all that is necessary is to save the prevalence at the relevant point in the model, in this case just after stabilization). It is assumed that the illness rate is proportional to the prevalence in servings, and the uncertainty distribution for the ratio of illness rate to prevalence calculated (that is, the illness rate per contaminated serving). For type A CPE$^+$ *C. perfringens* cells (vegetative plus spores), this gives 95th and 99th percentiles of the distributions of illnesses per contaminated serving of 8.9×10^{-4} and 13.8×10^{-4} illnesses per contaminated serving, respectively (Fig. 8). For total *C. perfringens* cells, it gives 95th and 99th percentiles of the distributions of illnesses per contaminated serving of 5.5×10^{-4} and 8.3×10^{-4} illnesses per contaminated serving, respectively (Fig. 9). Applying to the ALOP gives the POs shown in Table 2 for vegetative cells plus spores of *C. perfringens* just after stabilization (the values for the POs are higher than
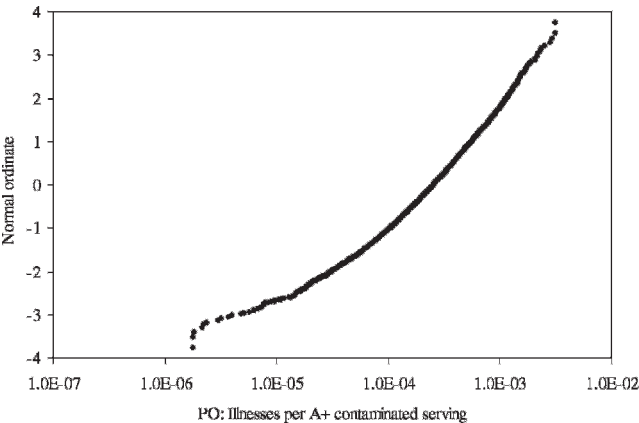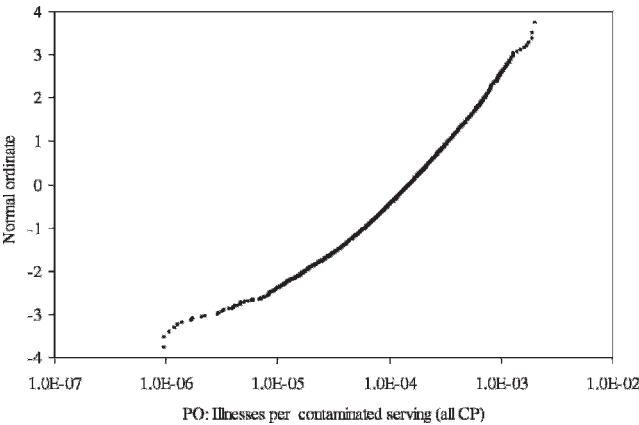
the corresponding values for the FSOs in Table 2, primarily because of the effect of cooking a large fraction of the hot dogs consumed—such cooking will generally kill most or all of any *C. perfringens* present).

(ii) A PO based on a fixed food mass. The preceding discussion has been based on the prevalence in hot dog servings. This is a natural unit of exposure for the risk assessment and corresponds to how people are exposed. However, it does not represent a PO that is particularly practical for implementation of an MC, since it would require random sampling and analysis of samples with weights chosen from the distribution of hot dog serving size, and detection of both ungerminated spores and vegetative cells within that entire sample.

For evaluation of sampling plans, the actual sample weight that is analyzed for cells is required. The only important quantity is the actual amount of originally sampled material that is analyzed for *C. perfringens,* taking into account any dilution or concentration steps; the originally selected sample size is irrelevant. For this demonstration, it is assumed that the sampling procedure can measure vegetative cells in 1 g of sample.

For the evaluation of a PO, therefore, it is assumed that the illness rate is proportional (but with uncertainty) to the vegetative cell prevalence in 1-g samples, and the distribution of the ratio of illness rate to prevalence in 1-g samples calculated within the risk assessment model, just as for the prevalence in servings discussed earlier. This required adding a component to the risk assessment model to calculate *C. perfringens* incidence in such a 1-g sample, in parallel with the calculation for a whole serving.

Figure 10 shows the uncertainty distribution for the ratio of illnesses to contaminated 1-g samples (i.e., illness rate divided by prevalence of vegetative cells in 1-g samples). The 95th and 99th percentiles of these distributions are for type A CPE$^+$, 6.2×10^{-3} and 9.1×10^{-3} illnesses per contaminated sample, respectively, and for total *C. perfringens,* 1.1×10^{-3} and 1.6×10^{-3} illnesses per contaminated sample, respectively. From these, POs may be derived as before for the prevalence in 1-g samples, as shown in Table 3.

FIGURE 8. *Uncertainty distribution for the ratio of illness rate to* Clostridium perfringens *contamination rate by type A CPE$^+$ vegetative cells plus spores just after stabilization.*

TABLE 2. *POs for the two ALOP selections and two levels of certainty*

ALOP illnesses/ million servings	Level of certainty (percentile)	Prevalence in hot dog servings of:	
		Type A CPE$^+$ C. perfringens (%)	Total C. perfringens (%)
21	95th	2.36	3.84
	99th	1.52	2.52
13	95th	1.47	2.39
	99th	0.95	1.57

The large difference between the columns for type A CPE$^+$ and total *C. perfringens* in Table 3, compared with the much smaller difference in Tables 1 and 2, is due to the extreme nature of the concentration distribution of *C. perfringens* estimated in the risk assessment.

Evaluation of an MC for *C. perfringens* at the plant gate. (i) Relation of PO and MC. The PO evaluated earlier is a microbiological specification that contributes to an ALOP. To meet such a specification requires an acceptability criterion, an MC, for a food product or food lot. An MC consists of various components (see §2.1 in *(1)*); two of concern here are (i) a plan defining the number of field samples to be taken and the size of the analytical unit, and (ii) microbiological limits considered appropriate to the food at the specified point(s) of the food chain.

The appropriate microbiological limit is the PO obtained earlier, and in what follows, it should be recalled that the PO has, for *C. perfringens,* been evaluated in terms only of the prevalence of *C. perfringens*. In evaluations of other organisms, it might also be appropriate to take into account the level of the organism in food when designing sampling plans to demonstrate or evaluate compliance with the PO, or the effect of process controls in achieving the PO.

(ii) Design of a sampling plan. Evaluation of an MC involves designing a test program that can detect the prevalences shown in Table 3, with acceptable false-positive and -negative rates. The discussion here is primarily concerned with the sample sizes required, in order to evaluate the feasibility of any such MC. Sampling of hot dogs is assumed at random, with 1 g of any sample actually analyzed (this is the size of the analytic unit). It is assumed that the detection rate for vegetative cells is 100%.

The appropriate selection from Table 3 are prevalences of approximately 1.3% for total *C. perfringens,* or 0.22% for type A CPE$^+$ *C. perfringens*. Detection of type A CPE$^+$ *C. perfringens* first requires detection as *C. perfringens,* with additional tests for type A CPE$^+$, so it would be less efficient than just detecting total *C. perfringens*.

The false-positive and -negative rates allowable for the MC would likely depend on circumstances, depending on the stringency required to demonstrate compliance. The design of a sampling plan is standard, and requires specification of the increment in prevalence to be detected with some specified (high) probability, and specification of the (low) probability to falsely flag noncompliance.

Suppose that any prevalence of s or higher is to be detected with probability P_d or more, while any prevalence equal to or less than the PO of p must be declared noncompliant with probability P_n or lower. Then for the most efficient sampling plan it is required to find the smallest N and an R, $0 \leq R \leq N$ such that:

$$\sum_{r=R}^{N} \binom{N}{r} s^r (1-s)^{N-r} \geq P_d$$

$$\sum_{r=R}^{N} \binom{N}{r} p^r (1-p)^{N-r} \leq P_n \qquad (11)$$

For example, choosing $p = 0.013$ (the PO), $s = 0.026$ (double the PO), $P_d = 0.9$, and $P_n = 0.1$ gives the minimum number of samples N as 727, with $R = 14$ or more indicating noncompliance.

DISCUSSION

With this demonstration, we show that evaluating ALOPs corresponding to current practices, and FSOs, POs, and MCs corresponding to such ALOPs, is practical when using a suitable QMRA. A QMRA that accounts for both variability between food servings and uncertainties is essential to the methodology described here. The *C. perfringens* risk assessment model *(3)* is such a 2-D model (implemented by using Monte Carlo methods) that is suitable. In addition to making the food safety approach transparent and quantifiable *(16),* given such an existing model, the approach described here is practical for linking public health outcomes to food safety measures and objectives.

Preliminary attempts (not described here) using just the summary results of the *C. perfringens* risk assessment and assumptions about the shape of various distributions (e.g.,
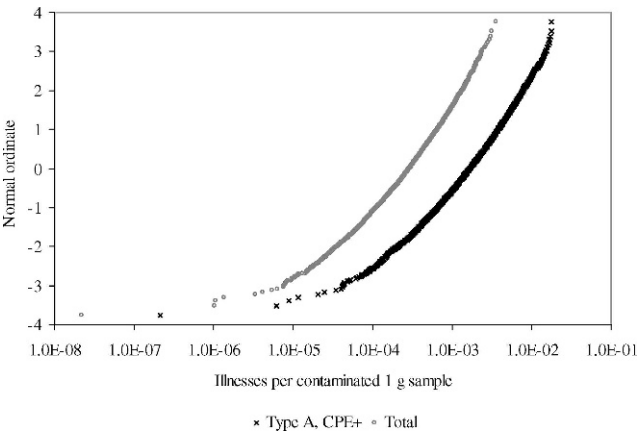

FIGURE 10. *Uncertainty distribution for the ratio of illnesses to vegetative cell–contaminated 1-g samples.*

TABLE 3. *POs for 1-g samples for the two ALOP selections and two levels of certainty*

ALOP illnesses/ million servings	Level of certainty (percentile)	Vegetative cell prevalence in 1-g samples of:	
		Type A CPE$^+$ *C. perfringens* (%)	Total *C. perfringens* (%)
21	95th	0.34	2.00
	99th	0.22	1.29
13	95th	0.21	1.24
	99th	0.13	0.80

assumptions about log normal or uniform distribution shapes) were inadequate to allow adequate definition of FSOs or POs. The actual distributions differed substantially from such idealizations, and the results depended critically on those differences, so the full QMRA was required. The QMRA must be available to be modified, and the analyst performing the modifications needs to be completely familiar with it (including down to the code level, assuming a computer model). For example, it is highly unlikely that a risk assessment model will have been designed to output all the results required during the procedures summarized above, and considerable experimentation may be necessary to evaluate suitable metrics for FSOs and POs. The approach is therefore not necessarily simple to implement.

Further, the level of detail required for setting ALOPs, FSOs, and POs for a particular food type is likely to be higher than required for evaluating a whole industry for one organism. Thus, more detailed information may be necessary than is available in a whole-industry assessment. In particular, the representativeness of the measurements used and the assumptions made for the whole-industry assessment may be more questionable when applied in detail to particular food types.

The approach demonstrated here is entirely general, provided a 2-D QMRA (i.e., one that separates uncertainty and variability) is available. For example, the same approach may be used to develop a PO at any point within the food chain, provided only that the QMRA suitably models the food chain and provides access to that point in the food chain within the model. However, the risk assessment model may impose limitations—the *C. perfringens* model could not be used to evaluate process controls, for example, since it does not model them. To be fully useful, the QMRA used to link ALOPs with FSOs or POs must be developed with this risk management objective during the planning and scoping of the risk assessment. Other examples attempt to link the QMRA, these FSO and PO metrics, and risk management options with other organisms, e.g., *Listeria (8, 10, 11)*.

While the approach demonstrated is general, the choices made in this particular demonstration are specific to *C. perfringens*, which has characteristics substantially different from other foodborne disease organisms. Evaluation of FSOs and POs for other organisms will be sufficiently different that drawing general conclusions based on the specific results obtained in this one demonstration (e.g., the use of prevalence alone for a PO; see above) may not be advisable. Further, while the methodology discussed here is general, it may not cover all possibilities—care must

be taken in specific cases to adapt and extend as necessary. Finally, if a suitable QMRA model is available, in principle, it may not be necessary to evaluate FSOs, although FSOs may be desired or required for other purposes. A direct evaluation of a PO corresponding to the ALOP is straightforward—attempting to base the PO on the FSO rather than the ALOP in such circumstances (*(12)* also draws this conclusion).

ACKNOWLEDGMENT

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